Stagonospora nodorum: From Pathology to Genomics and Host Resistance

Richard P. Oliver,[1] Timothy L. Friesen,[2] Justin D. Faris,[2] and Peter S. Solomon[3]

[1]Australian Center for Necrotrophic Fungal Pathogens, Curtin University, Perth WA 6845, Australia; email: richard.oliver@curtin.edu.au

[2]U.S. Department of Agriculture, Agricultural Research Service, Cereal Crops Research Unit, Fargo, North Dakota 58102-2765

[3]Research School of Biology, The Australian National University, ACT 0200, Australia

Annu. Rev. Phytopathol. 2012. 50:23–43

First published online as a Review in Advance on May 1, 2012

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

This article's doi: 10.1146/annurev-phyto-081211-173019

0066-4286/12/0908-0023$20.00

Keywords

Phaeosphaeria, *Septoria*, *Leptosphaeria*, glume, Dothideomycetes, Pleosporales, necrotroph

Abstract

Stagonospora nodorum is a major necrotrophic pathogen of wheat that causes the diseases *S. nodorum* leaf and glume blotch. A series of tools and resources, including functional genomics, a genome sequence, proteomics and metabolomics, host-mapping populations, and a worldwide collection of isolates, have enabled the dissection of pathogenicity mechanisms. Metabolic and signaling genes required for pathogenicity have been defined. Interaction with the host is dominated by interplay of fungal effectors that induce necrosis on wheat lines carrying specific sensitivity loci. As such, the pathogen has emerged as a model for the Pleosporales group of pathogens.

INTRODUCTION

Model species have played revolutionary roles in the development of major biological concepts. Species were initially chosen for speed of reproduction, stability of phenotype, and ease of cultivation. Once communities of researchers adopt a model species, the shared (mostly genetic) resources further hasten progress, attracting more effort to the models. In the case of plant pathology, two species—both host and microbe—should possess a model's resources. The research community has focused on the models provided by tractable bacterial pathogens and *Arabidopsis*, and this has enabled rapid advances that have proven to be broadly applicable, leading to the current consensus on the molecular basis of biotrophic (viral, bacterial, fungal, and nematodal)/host interactions (19, 49).

The standard definition of necrotrophic pathogens is that they derive their energy from dead host cells (54, 75) in contrast to biotrophs that derive energy from living cells. This definition hides obvious differences in pathogenic strategy between fungal pathogens such as *Magnaporthe grisea*, *Botrytis cinerea*, *Fusarium graminearum*, and *Mycosphaerella graminicola*. These pathogens, all of which can be described as necrotrophs (75), significantly differ in pathogenic properties. *B. cinerea* rapidly invades and kills host cells, whereas the other species exhibit extended asymptomatic (and arguably biotrophic) phases followed by a switch to host-cell killing. Host-cell death is generally attributed to a cocktail of nonspecific toxins and cell wall–degrading enzymes produced during infectious growth. As a result, no consensus on the nature of necrotrophy has emerged.

Stagonospora nodorum is a major pathogen of wheat that has emerged as a model for a specific class of necrotrophic pathogens, the Pleosporales. A combination of resources—methods for functional gene characterization, a propensity to secrete specificity factors into culture filtrates, a worldwide collection of isolates, genetically characterized host-mapping populations, and the genome sequence—has allowed us to construct a working model of pathogenic evolution based on necrotrophic effectors. This model is being successfully adopted by breeders to advance more resistant cultivars. Furthermore, the model provides a framework for the study of other Pleosporales. The studies highlight several important features of chromosomal evolution and paint a picture of genomic flexibility. The purpose of this review is to highlight developments, mainly since 2003, that underpin this novel consensus.

TAXONOMY, INCIDENCE, AND IMPORTANCE

S. (syn. *Phaeosphaeria*, *Septoria*, *Leptosphaeria*) *nodorum*, cause of wheat S. nodorum leaf and glume blotch, has been reviewed most recently in 2006 (93, 104). It falls within the class Pleosporales in the order Dothideomycetes (88), a newly recognized taxon that broadly replaced the Loculoascomycetes. Dothideomycetes contains many important phytopathogenic genera, including *Pyrenophora*, *Alternaria*, *Cochliobolus*, *Leptosphaeria*, and *Corynespora* within the Pleosporales, as well as *Venturia*, *Passalora* (*Cladosporium*), and *Mycosphaerella*. The evolutionary distances between these groups and other filamentous fungi are very large. The last common ancestor is dated at 400 mya, much older than their angiosperm hosts (45), which diverged approximately 80 mya.

S. nodorum is taxonomically distant from *M. graminicola* (syn. *Septoria tritici; Zymoseptoria tritici*) (cause of Septoria leaf blotch) but closer to *Pyrenophora tritici-repentis* (cause of tan spot). These pathogens (and others) have similar symptoms and frequently coinfect wheat. It is easy to misidentify lesions, although spore morphology and DNA-based tests can be used for diagnosis (77).

S. nodorum blotch (SNB) is a major disease of durum and common wheat in many parts of the world. In Australia, it is largely restricted to western Australia, where it reportedly causes damage costing an average of >$100 million per annum (approximately 5%

of total yield), making it second only to tan spot in cost of damage (73). It is unclear why the disease is scarcely seen in eastern Australia, where conditions would appear to be broadly similar. Western Australia's wheat belt has more consistent rainfall than eastern Australia, which suffered a run of drought years during the survey period of 2000–2008. *S. nodorum* is considered one of the most damaging pathogens in North America (26) and is reported in South America, South Asia, and North Africa.

In Europe, the most complete data set showing prevalence of the disease originates from Rothamsted. The Broadbalk collection of wheat samples from Rothamsted Research Station has been used to estimate the incidence of *S. nodorum* from 1844 to 2003 (11, 90). *S. nodorum* was the predominant disease until approximately 1970, when it was overtaken by *M. graminicola*, a trend confirmed more broadly in Europe. The reasons for the decline of *S. nodorum* are obscure. Bearchell et al. (11) point to a correlation with declining sulfur pollution levels. However, no functional link has been made, and sulfur pollution levels are very low in the western Australian *S. nodorum* hot spot. The partial replacement of *S. nodorum* by *M. graminicola* is likely due to a combination of factors, including fungicide use and the introduction of cultivars susceptible to Septoria leaf blotch caused by *M. graminicola* (7).

Systematic surveys of disease incidence are lacking in nearly all parts of the world. We must rely on essentially anecdotal reports. Despite these limitations, it is clear that *S. nodorum* remains regularly detected in nearly all wheat-growing areas and is one of the top three pathogens when quantified by economic loss.

GENOMICS

The genome of *S. nodorum* was sequenced in 2005 and published in 2007 (41). It is no exaggeration that this development had a revolutionary impact on our understanding of this pathogen. Before 2007, only limited genomic information was available. A handful of genes had been characterized, and some data on repetitive elements were available (50, 51, 69, 70). A small collection (~1,000) of expressed sequence tags (ESTs) were in use (92, 95, 99, 100). Pulsed-field gel electrophoresis had suggested the genome consisted of 14–19 chromosomes (25). Conventional genetic analysis had been achieved but could not be reproduced in other laboratories (63).

The first fungal genomes to be sequenced were the intensively studied model species, such as the yeasts *Saccharomyces*, *Candida*, and *Schizosaccharomyces* and the filamentous *Aspergillus nidulans* and *Neurospora crassa*. Among pathogens, sequences were published only for *Magnaporthe grisea* and *Ustilago maydis* (45). Genome sequencing was restricted to organisms supported by large scientific communities. These communities not only were needed to raise the substantial funds required but also were thought necessary to analyze the data. The Australian Grains Research and Development Corporation, a levy-funded and largely farmer-controlled organization, agreed to fund the sequencing of the *S. nodorum* genome in 2004. The Australian Centre for Necrotrophic Fungal Pathogens (ACNFP) organized the process and commissioned the Broad Institute to carry out the sequencing and some preliminary bioinformatic analyses. The *S. nodorum* genome was the first Dothideomycete genome to be published (although the community was well aware of commercial projects) and remained so until 2010 (28). The analysis was undertaken by just two labs, one for the nuclear and one for the mitochondrial genome, with taxonomic input from a third. One could say that never in the field of genomics was so much sequenced by so few to such effect.

The genome sequence was obtained by a 10x Sanger process using 1-, 4-, and 40-kb plasmid libraries of the Australian isolate SN15. The initial assembly identified 109 scaffolds, of which two proved to be the mitochondrial genome. The 107 nuclear scaffolds added up to 37.21 Mbp. Bioinformatic analysis of the assembly was hampered by the lack of closely related comparator data. An unexpectedly high total of 16,597 genes was predicted and released

on the Broad Institute Web site (**http://www.broadinstitute.org/annotation/genome/stagonospora_nodorum/MultiHome.html**). This number was substantially higher than typically found in the other filamentous fungi. A larger EST data set was created and used to refine the gene predictions. The 2007 genome paper (41) listed 10,792 genes supported by the EST data set and a more stringent gene-prediction software. Since then, extensive proteomic, transcriptomic, homology, and resequencing data have been used to refine the gene set to a current estimate of 12,382 nuclear genes (16, 45, 47, 113, 114). The current gene list can be obtained from the ACNFP (**http://acnfp.curtin.edu.au/research/**).

The initial set of gene models was used to create a custom microarray that was probed with transcripts from the fungus grown vegetatively and during sporulation in vitro and in planta (47). Positive signals of expression were obtained for 12,281 gene models, which provided strong evidence of the genes' validity. Numerous genes were differentially expressed, which was particularly apparent among genes expressed during infection. A disproportionate number of these genes (approximately 60% compared with approximately 30% for the genome as a whole) had no meaningful homologs in databases and proved to be a source of candidates for effector genes.

Considerable effort has also gone into studying the proteome of *S. nodorum*. Both gel- and non-gel-based proteomics techniques have been established and exploited, including relative quantitative proteomics using *i*TRAQ (isobaric tag for relative and absolute quantification) (16, 17, 113, 115). The resulting peptide data have been used to refine the gene annotation (16). The identified peptides validated 2,134 gene models, of which 62% had no prior experimental support. A comparison of the peptide data to the six-frame translation of the genome sequence resulted in many gene models being revised or newly identified. These included 144 genes with exon frameshifts, 604 genes with extended exons, and 3 entirely new genes. This approach, termed proteogenomics, also resulted in the identification of 44 potential new genes residing within unassembled regions of the genome.

The application of metabolomic techniques to *S. nodorum* has revealed multiple aspects of its interaction with wheat. Metabolomics is the term used to describe the nontargeted identification and quantification of the metabolome. Three different metabolomics platforms, gas chromatography–mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) spectroscopy, have been used to characterize *S. nodorum* and wheat (48, 60, 61, 94, 98, 116). These techniques are complementary and have proven to be powerful tools in dissecting the infection of wheat by *S. nodorum* and also the phenotype of some significant *S. nodorum* mutants, particularly those involved in primary metabolism and signal transduction, as discussed below.

COMPARATIVE GENOMICS: MESOSYNTENY

Comparisons of genome sequences of animals and plants have shown that species from within families generally share contiguous stretches of genes that are orthologous and colinear. Also, entire chromosomes in the different species are broadly colinear, albeit with some gaps and rearrangements. The former phenomenon has become known as microsynteny and the latter as macrosynteny (see 44 for references and for a discussion of synteny terminology). The filamentous fungi are an ancient and diverse group of species, with germ-line life cycles measured in days or weeks. This contrasts with plants and animals, where germ-line life cycles are measured in months, years, or even decades. Hence, it was not surprising when comparisons of fungal genomes completed before 2007 failed to find extensive evidence of either macro- or microsynteny. Even species from within a genus often lacked significant synteny.

Comparison of the synteny relationships of *S. nodorum* with other Dothideomycetes revealed a striking pattern of chromosomal

evolution that has been named mesosynteny. Whole-genome dot plots revealed that each chromosome (or scaffolds in the case of uncompleted genomes) contains genes that are predominantly located on just one chromosome in another species. However, unlike animals and plants, where genes are broadly in the same order and orientation, in the Dothideomycetes the order and orientation is extensively shuffled. Comparison of genomes in different filamentous fungal classes showed a weaker pattern that was better defined as degraded mesosynteny than as macrosynteny. No syntenic pattern was observed in comparison with yeasts.

A convincing explanation for mesosynteny has yet to emerge. The observation implies that interchromosomal rearrangements are rare but that intrachromosomal inversions are common on an evolutionary timescale. Possible explanations have focused on two phenomena associated particularly with filamentous ascomycetes: repeat-induced point mutations (RIP) and horizontal gene transfer.

REPEAT-INDUCED POINT MUTATION

RIP is described as a genome defense mechanism whereby duplicated sequences are mutated during meiosis (89). Although direct evidence of RIP is restricted to a handful of species, bioinformatic evidence suggests that it is ubiquitous in filamentous ascomycetes and rare or absent in other fungi (23). Bioinformatic analysis depends on the observation that the mutations are nonrandom. *S. nodorum* RIP appears to follow the pattern established in *N. crassa* whereby CpA dinucleotides are mutated to TpA (42, 43). Using an alignment approach, the repetitive fraction of the *S. nodorum* genome could be shown to be replete with evidence of RIP. Approximately 6% of the genome is repetitive and comprises 25 families of repeats (42, 43). Many of these families, including the rDNA repeat, show evidence of RIP. As expected, many of the repeat families appear to be relics of various types of transposons, although none appear to be intact in the SN15 genome assembly. Several studies indicate that the repetitive-element content of different *S. nodorum* strains is highly variable (18, 25, 50, 69, 70, 108). An unpublished study used a transposon trapping technique to identify three transposons in a U.K. isolate (81). Relics of these transposons could be found in the SN15 genome, indicating that RIP has successfully disabled these elements.

Because RIP involves systematic conversion of CpA dinucleotides into TpA, predicting the sequence prior to RIP is possible (43). An algorithm to achieve this was used to predict the function of the repetitive elements in SN15. Five new classes of transposons and four gene repeats were discovered using this process. RIP has been linked to the evolution of effector genes in *Leptosphaeria maculans* (39, 84, 121), but there is no compelling evidence that effector genes in *S. nodorum* have been mutated by a RIP-like process.

The role of RIP in maintaining mesosyntenic relationships is one of guilt by association; both phenomena—RIP and horizontal gene transfer—are hallmarks of filamentous ascomycetes. We can speculate that by destroying regions of homology between chromosomes, RIP reduces the frequency of translocations. However, it is not clear why this should not also apply to inversions.

HORIZONTAL GENE TRANSFER

The growing catalog of sequenced fungal genomes has provided convincing evidence that a large proportion of the genes has been acquired from other species at some time—ranging from decades to hundreds of millions of years ago (83, 87, 123). Approximately 20% of genes in *S. nodorum* have no obvious homologs in closely related species; this can be regarded as preliminary evidence that they have been horizontally acquired. In several cases, specific pathogenicity determinants appear to have been horizontally acquired (3, 37, 80). Indeed, the emergence of tan spot as a new disease of wheat in the twentieth century has been convincingly linked to the horizontal transfer of

ToxA from *S. nodorum* to *P. tritici-repentis* (37). Furthermore, *ToxA* was likely acquired horizontally by *S. nodorum* in the past 300 years (111). Horizontal gene transfer can therefore be seen not as a rare and ancient event but as a regular mechanism to acquire new genes. It can also be seen as a way for genes to acquire new genomic hosts.

Again, the link to mesosynteny is one of guilt by association. The suggestion is that fungi frequently form anastomotic connections, allowing the transfer of genes between nuclei. If the process involved the creation of a dikaryon followed by chromosome loss, the chromosome containing the relevant new gene could be retained in place of its homolog without loss of essential functions.

POPULATION GENETICS AND BIOGEOGRAPHY

S. nodorum is a pathogen of wheat both in Mediterranean climates, in which short-season cultivars are grown and are characterized by long, hot summers, and in temperate regions, in which long-season, spring-sown, and autumn-sown cultivars are grown. Survival of inocula on stubble is an essential feature for pathogenicity in Mediterranean climates, and this is linked to ascospore infection (13) (**Figure 1**). Asexual spores are adequate for seed-borne and foliar inocula. *S. nodorum* is a heterothallic species. The ratios of the different mating types have been studied in populations from all over the world and were found not to differ significantly from the expected 1:1 (12, 15, 72, 96, 107). This indicates that sexual reproduction is frequent under all conditions.

The biogeography of *S. nodorum* has been studied using the mitochondrial genome and nuclear simple sequence repeats and a worldwide collection of populations (105, 106, 108–111). The center of diversity was the Fertile Crescent. Patterns of variation were highest in populations from Asia and Europe and lowest in Australia, consistent with the pathogen having been spread along with the cultivation of wheat. Overall, there were low levels of differentiation between continents, suggesting rather large populations were spread to each continent. The low level of variation in Australia suggests that quarantine methods and the physical separation have combined to prevent significant recent immigration.

FUNCTIONAL GENOMICS TOOLS

Polyethylene glycol–mediated transformation techniques were developed for *S. nodorum* in the late 1980s (24). Homologous recombination rates of 1% to 5% were typically achieved so that most genes selected for targeted disruption could be inactivated (92, 93, 95, 100). Within the past 5–6 years, the transformation of knockout constructs created by polymerase chain reaction (PCR) as well as by split-marker constructs with homologous ends dramatically increased the rate of homologous recombination from 30% to 100% (97). The genome sequence enabled the design of primers to detect gene ablation events. Recently, Feng et al. (33) developed a *KU70* disruption strain of *S. nodorum*. The Ku70 protein, involved in the nonhomologous end-joining double-strand DNA break pathway, was identified and deleted. The inactivation of the *KU70* gene in many fungi has resulted in a significant improvement in homologous recombination rates. The resulting *S. nodorum* strain showed no apparent phenotypic differences compared with the wild type and resulted in a significant increase in the rate of homologous recombination.

PATHOGEN PRIMARY METABOLISM PLAYS AN IMPORTANT ROLE DURING INFECTION

A series of mutants has been created over the past 10 years that have highlighted metabolic pathways of *S. nodorum* required during the infection of wheat; the role of these genes in the life cycle is illustrated in **Figure 1**. Starting on the leaf surface, inactivation of the malate synthase gene (*Mls1*) demonstrated that the fungus was unable to germinate in the absence

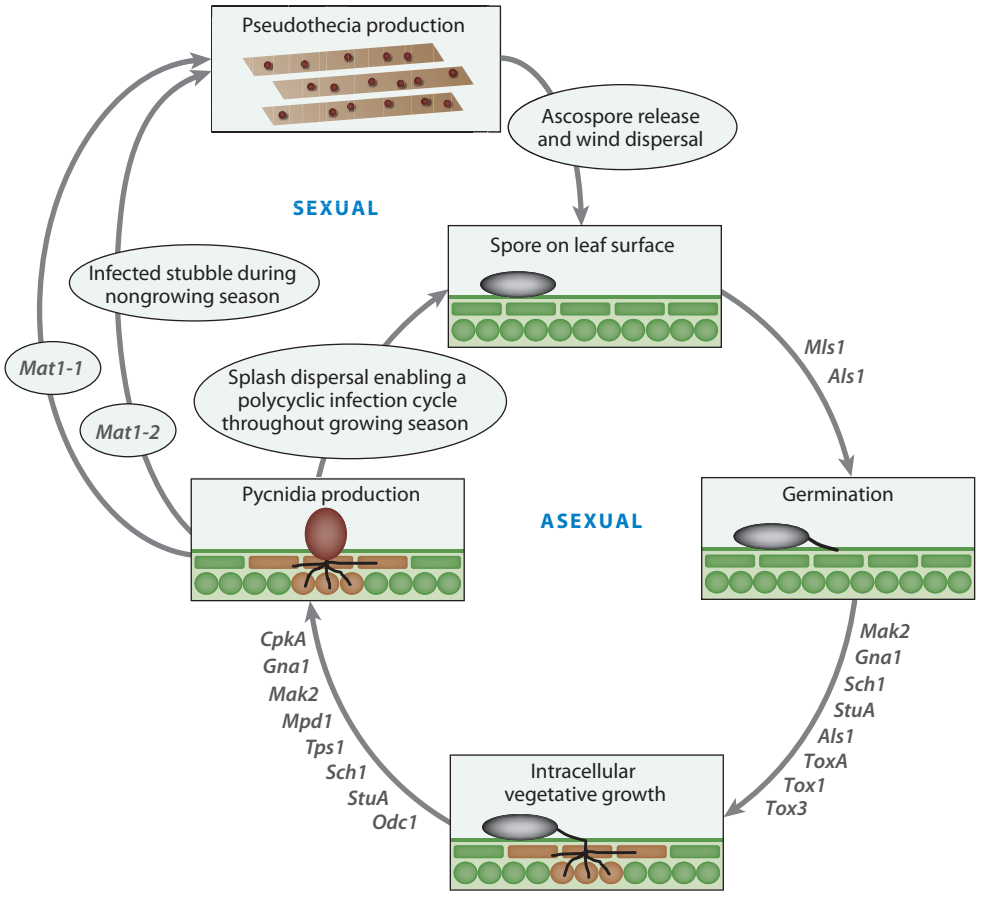

Figure 1

The sexual and asexual life cycle of *Stagonospora nodorum* annotated with genes required at different stages.

of an exogenously supplied carbon source (92). Malate synthase is an enzyme in the glyoxylate cycle that catalyzes the condensation of glyoxylate and acetyl CoA to form malate. The acetyl CoA is generated through the breakdown of internal triacylglycerol stores, and the only way to synthesize sugars from this carbon is through the synthesis of oxaloacetate in the glyoxylate cycle. The inability of the *mls1* strains to germinate strongly suggests that internal lipid stores supply the carbon for germination. Similar studies in other pathogens have shown contrasting results. The disruption of isocitrate lyase in the related Dothideomycete *L. maculans* showed a similar phenotype (46). However, comparable studies in the rice blast pathogen *Magnaporthe oryzae* have suggested that glycogen is the internal store of carbon for germination (117), implying that different pathogens have evolved independent metabolic requirements to suit their pathogenic life cycle.

δ-aminolevulinic acid (ALA) is a key intermediate in the synthesis of porphyrins. Strains lacking ALA synthase were able to germinate on the leaf surface and in vitro but unable to progress any further (91). Supplementation of the infection with exogenous ALA partially restored pathogenicity, as did infection through

wounded tissue, suggesting that the pathogen can utilize a host-derived source of ALA during infection.

The transcriptomic and metabolomic data sets were cross referenced in both in planta and in vitro samples (47). One unfamiliar metabolite, β-alanine, was found abundantly and focused attention on the pantothenate pathway. Pantoate-β-alanine ligase, encoded by *Pbl1*, is the first committed enzyme in the biosynthesis of CoA, an essential cofactor in central metabolism. As expected, ablation of the gene rendered the strain auxotrophic for pantothenate, but it was entirely unexpected that this strain would have unaltered pathogenicity on wheat. These findings imply that the fungus can obtain CoA (or a precursor after pantothenate) from the host. The apparent ability of *S. nodorum* to take up complex molecules like ALA and CoA may open up new avenues of disease control.

S. nodorum is a polycyclic pathogen and requires multiple rounds of asexual sporulation to cause major yield losses. Consequently, considerable effort has been devoted to understanding how *S. nodorum* completes it pathogenic life cycle. One such study revealed that levels of mannitol significantly increased at the onset of pycnidial maturation. A series of mutants were created lacking mannitol dehydrogenase (Mdh1) and/or mannitol 1-phosphate dehydrogenase (Mpd1) (98, 101, 102). These studies showed that the ability to synthesize mannitol is critical for the pathogen to sporulate. Although the exact role of mannitol during asexual sporulation is yet to be determined, phenotypic assays showed that mannitol appears to have no role in combating oxidative or osmotic stress. A similar study found that trehalose also accumulated during sporulation. Mutants of *Tps1*, encoding trehalose 6-phosphate synthase, were unable to sporulate, indicating an essential role for trehalose metabolism (61). However, unlike *M. oryzae*, mutants in *S. nodorum* suffered no growth defect on nitrate, implying that Tps1 does not have the intricate NADPH-sensing role identified in the rice blast pathogen (125, 126).

SIGNAL TRANSDUCTION AND GENE REGULATION

As with the dissection of primary metabolism, functional genomics has also highlighted the critical roles of *S. nodorum* signal transduction during its interaction with wheat. Strains of the pathogen harboring inactive copies of a MAP kinase (*Mak2*) and calcium/calmodulin-dependent protein kinases (*Cpk1*, *Cpk2*, and *Cpk3*) have shown that these pathways regulate a variety of cellular processes and phenotypes, including sporulation, pathogenicity, and protection against various stresses (97, 103). These strains have provided a valuable tool in understanding pathogenicity. This was particularly so for the *S. nodorum* strain, *gna1*, which harbored a disrupted copy of the G-α subunit gene, *Gna1* (99). As with the other signaling mutants, the *gna1* strain showed multiple abnormal phenotypes, including reduced pathogenicity, reduced secreted protease activity, and an inability to asexually sporulate. A comprehensive proteomics screen was undertaken to investigate these phenotypes further and also to determine the downstream targets of Gna1 signaling that played a role in these phenotypes (17, 113, 114). Multiple proteins were identified that displayed either increased or decreased abundance in the absence of Gna1. One such protein was Sch1, an unidentified short-chain dehydrogenase, which was absent in the *gna1* background. Subsequent analysis of *S. nodorum* strains lacking *Sch1* showed that it was required for maturation of pycnidia during asexual sporulation (114). Microscopic analysis showed that pycnidia began to differentiate in the *sch1* strains but appeared to prematurely abort, leading to an undifferentiated wall structure and few viable pycnidiospores.

The characterization of the *sch1* strains described above demonstrated a clear role for Sch1 in asexual sporulation, although its function remained unknown. As it was predicted to encode a short-chain dehydrogenase, a nontargeted GC-MS metabolomics screen was undertaken to determine if the function of Sch1 could be ascertained (116). A comparison

of the *sch1* mutant and wild-type metabolomes revealed a massive increase in the abundance of a compound that eluted at 45.57 min. The compound was identified as the mycotoxin alternariol. This demonstrated for the first time that *S. nodorum* harbors the metabolic potential to synthesize a primary mycotoxin. This finding has raised questions regarding the safety of *S. nodorum*–contaminated grain, and studies are now under way to determine the relevance of this finding under field conditions.

The role of key transcription factors has also been assessed using a combination of techniques. *StuA* belongs to a class of transcription factors unique to the ascomycetes that regulate developmental differentiation. Disruption of *StuA* showed that the gene played a role in asexual sporulation and pathogenicity (48). The *stuA* mutants had major primary metabolic perturbations. Nearly all sugars and sugar alcohols were much more abundant in the *stuA* mutants, whereas amino acids and other metabolite classes, such as polyamines, were downregulated. These data show that *StuA* plays a central role in primary metabolic regulation and, along with the identified role of mannitol in asexual sporulation, also provide further evidence for the critical role of primary metabolism in asexual sporulation.

FUNGICIDAL CONTROL

S. nodorum can be efficiently controlled by a wide range of fungicides in current use. Reports of fungicide resistance are rare, probably because of the low prevalence of the disease in Europe with its long history of intensive fungicide use. Fungicide use has significantly expanded in Australia and other lower yielding countries in the past decade, so we should remain vigilant (73). Widespread resistance has been detected in Scandinavia to the QoI fungicide azoxystrobin and was associated with the G143A mutation found in the mitochondrial *cytB* gene (14). These pathogens were susceptible to normal levels of triazole fungicides. Two copies of the triazole target gene *cyp51*, were found in the SN15 genome, although only one was expressed (47).

HOST-SELECTIVE TOXINS TO NECROTROPHIC EFFECTORS: THE INVERSE GENE-FOR-GENE MODEL

Before the advent of molecular markers, dissecting SNB resistance was difficult because the trait was consistently observed to be quantitative and the underlying mechanism was not understood. Resistance proved to be polygenic, and improvements in resistance were based on pragmatic field screens. Molecular markers allowed new strategies for characterizing resistance to quantitative diseases. Markers could be used to detect quantitative trait loci (QTLs) associated with resistance, although the size of the wheat genome and the scarcity of markers hampered progress. QTLs for SNB resistance have been identified on almost every wheat chromosome (128). In 2004, the first host-selective toxin (HST) interaction was identified in the SNB system (58, 59), and this opened the possibility that disease resistance loci were determined by HST–host gene interactions that conferred susceptibility. A model has emerged whereby the pathogen produces (some of the) several necrotrophic effectors [formerly called host-specific or host-selective toxins named SnToxN, encoded by genes, *SnToxN*; now called necrotrophic effectors (NEs)], each of which interacts with a wheat susceptibility gene (named *SnnN*). Wheat cultivars differ in their complement of susceptibility genes. The severity of the disease is a function of the number and identity of matching effectors and susceptibility genes. This model can be regarded as an inverse gene-for-gene system (see **Table 1**).

SnTox1-*Snn1*

The SnTox1-*Snn1* interaction was the first interaction to be characterized. SnTox1 was originally shown to be a secreted protein of 10–30 kDa (58). Recently, the gene was cloned and the SnTox1 protein was found to be

Table 1 *Stagonospora nodorum* **necrotrophic effectors and host sensitivity/disease susceptibility loci**

Necrotrophic effector	Protein size/kD	Number of cysteines	Prevalence in pathogen populations	Mode of action	Host sensitivity gene	Wheat chromosome arm	Markers	Maximum variation explained	Status of host gene
SnTox1	9	16	85%	Light dependent	*Snn1*	1BS	*Xpsp3000*, *Xfcp618*, *Xfcp619*, *Xfcp624*	58%	Fine mapped
SnToxA	13.2	2	36%	Light dependent; disruption of photosynthesis	*Tsn1*	5BL	*Xfcp623*, *Xfcp394*, *Xfcp620*, *Xfcp1*, *Xfcp2*	95%	Cloned
SnTox2	7–10	?	?	Light dependent	*Snn2*	2DS	*XTC253803*, *Xcfd51*, *Xcfd56*	47%	Mapped
SnTox3	19	6	59%	Not light dependent	*Snn3-B1*	5BS	*Xcfd20*, *Xgwm234*	18%	Mapped
				Not light dependent	*Snn3-D1*	5DS	*Xbarc130*, *Xcfd18*, *Xhbg337*, *Xgwm190*	100%	Fine mapped
SnTox4	10–30	?	?	Light dependent	*Snn4*	1AS	*XBG262267*, *XBG262975*, *Xcfd58*	41%	Mapped

10.3 kDa (57). The SnTox1-*Snn1* interaction accounts for as much as 58% of the disease variation (59). The *Snn1* gene was mapped to the distal end of the short arm of wheat chromosome 1B (58), where it was shown to lie within a gene-rich region that is poorly conserved in rice (82). The locus was narrowed to a small chromosomal segment containing two members of the nucleotide binding site–leucine rich repeat (NBS-LRR) class of genes considered to be good candidates for *Snn1*. Also, tightly linked PCR-based markers suitable for marker-assisted selection against *Snn1* have been developed (**Table 1**).

SnTox1 was identified by scanning the genome sequence for genes with characteristics common to small, secreted effector-like proteins (57). Genes from this list were expressed in a yeast expression system, and the expressed proteins were infiltrated into diagnostic wheat lines (57). The protein produced by SNOG_20078 specifically induced necrosis on wheat lines harboring *Snn1*. Gene transformation into a strain lacking virulence on *Snn1* wheats and gene knockouts verified that indeed SNOG_20078 was the *SnTox1* gene. Transformation of an avirulent strain resulted in the ability to cause disease on *Snn1* wheat, and the *Snn1* gene was shown to be responsible for disease susceptibility. Also, reaction to necrosis induced by culture filtrates of these strains mapped to the *Snn1* locus. Conversely, deletion of SNOG_20078 in virulent strains resulted in mutants that lost the *Snn1* QTL as well as the secreted necrosis-inducing factor.

The *SnTox1* gene was identified in approximately 85% of a worldwide collection of *S. nodorum* isolates. *SnTox1* expression accelerated from 6 to 72 hours post inoculation (hpi) but increased dramatically from 24 to 72 hpi, which coincides with the early stages of infection and the appearance of necrosis (57).

The mature protein is 100 amino acids following cleavage of a signal peptide. Remarkably, it contains 16 cysteine residues, all involved in disulfide bridges necessary for the activity/stability of the SnTox1 protein. The C terminus has similarity to plant-specific chitin-binding domains, indicating that SnTox1 may have multiple roles.

The mode of action of SnTox1 is still under study. *SnTox1* and *Snn1* are required for host cell penetration and cell death. Necrosis requires light, as has been found in other NE interactions (2, 35, 66). However, when dark-inoculated plants were put back into a normal light cycle, the pathogen was able to penetrate and cause disease, showing that light was necessary for penetration and continued proliferation of the pathogen.

The NEs produced by *S. nodorum* appear to subvert the normal resistance response to induce necrosis that is exploited by the pathogen. H_2O_2 production after pathogen inoculation and SnTox1 infiltration has been observed using diaminobenzidine staining. Early and extensive staining was dependent on both *SnTox1* and *Snn1*. Classical defense responses, including upregulation of PR-1-1, a thaumatin-like protein gene, and a chitinase were also observed during the infection process. Strong DNA laddering, a classical hallmark of apoptosis in plants and animals (27), was also observed in *Snn1* lines as early as 10 h post infiltration, with SnTox1 having the strongest laddering at the 36-h point, indicating programmed cell death is involved early in the infection process.

SnToxA-*Tsn1*

ToxA was first identified and described in *P. tritici-repentis* (52, 53, 118). ToxA is associated with pathogenicity (22, 119), and host sensitivity to the effector is associated with susceptibility to the fungus (36, 52, 53). The 13-kDa effector was successively purified by Ballance et al. (8), Tomás et al. (119), Tuori et al. (120), and Zhang et al. (129). The designation PtrToxA was adopted (21). PtrToxA is encoded by a single gene (9, 22) that encodes a 178–amino acid pre-proprotein with a signal sequence to target the protein for secretion and a prosequence for proper folding. Site-directed mutagenesis and peptide inhibition studies (64, 71) identified essential amino acids within potential casein kinase 2 phosphorylation sites

and a vitronectin-like RGD (arginine-glycine-aspartate) cell attachment motif. The three-dimensional structure of ToxA revealed that the RGD motif is present in a solvent-exposed loop (85).

ToxA is internalized, using the RGD motif, via the cytoplasm and the chloroplast, but only in sensitive (*Tsn1*) wheat genotypes (66, 67). Biolistic bombardment with a green fluorescent protein–ToxA fusion protein showed that both sensitive and insensitive genotypes underwent cell death. This suggests that both genotypes harbor the intracellular machinery for cell death caused by ToxA, but the differences lie in the ability of the effector to be internalized. ToxA recognition in a sensitive wheat host likely results in the internalization of ToxA, possibly via receptor-mediated endocytosis.

Sensitivity to ToxA is dominantly inherited and dependent on *Tsn1*. Anderson et al. (5) screened wheat cytogenetic stocks to show that insensitivity to ToxA was not conferred by a gene product per se, but by the absence of a gene for sensitivity. Faris et al. (29) mapped the gene conferring sensitivity to ToxA to the long arm of chromosome 5B in wheat and designated it *Tsn1*. Saturation mapping of the locus and chromosome walking yielded information regarding the evolution of the *Tsn1* genomic region and the development of new markers suitable for marker-assisted selection against *Tsn1* (**Table 1**) (40, 62, 130). The molecular cloning of *Tsn1* revealed a 1,490–amino acid protein consisting of resistance gene–like serine/threonine protein kinase, nucleotide binding (NB), and leucine-rich repeat (LRR) domains, all of which are required for ToxA sensitivity (31). Among cultivated wheats, ToxA-sensitive genotypes carry the *Tsn1* allele, whereas most ToxA-insensitive genotypes are nulls. Analysis of a large collection of cultivated and ancestral wheat accessions indicated that *Tsn1* likely arose in the diploid B-genome progenitor of modern polyploid wheat through a gene-fusion event that brought the protein kinase and NBS-LRR domains together in the same open reading frame. Therefore, *Tsn1* is unique to wheat, and full-length orthologs do not exist in other species.

Because *Tsn1* does not encode an integrin-like protein or an extracellular receptor-like protein, and the Tsn1 protein does not interact directly with ToxA [at least not in yeast (31)], it is highly unlikely that the Tsn1 protein is the ToxA receptor. Tsn1 may act as a guard for a cellular membrane–spanning ToxA receptor and, upon recognition of ToxA binding, may initiate ToxA import through signaling that results in receptor endocytosis. ToxA may also enter the cell by other means before being recognized by Tsn1 through the formation of a protein complex involving other partners.

The discovery of the lateral transfer of ToxA from *S. nodorum* to *P. tritici-repentis* (37) was followed by characterization of the *Tsn1*-ToxA interaction in the wheat–*S. nodorum* system and comparison with the wheat–*P. tritici-repentis* system. The *SnToxA* gene from SN15 shares more than 99% identity with the *PtrToxA* gene at the nucleotide level. Only 36% of *S. nodorum* isolates carry *ToxA* genes, and different populations vary from 2% to 100% (111). Genes for ToxA are highly polymorphic, in contrast to the monomorphism in *P. tritici-repentis*. *Tsn1* governed sensitivity to SnToxA as well as Ptr ToxA (56) and accounts for as much as 95% of the variation in SNB in segregating wheat populations (20, 30, 32, 34, 35, 37, 56). These studies included seedlings and adult plants, field and glasshouse studies, and durum as well as bread wheats.

Markers for *Tsn1* have been available for use by breeders for more than a decade and used to identify lines resistant to both SNB and tan spot. More recently, breeders in Australia in particular have used the expressed ToxA protein to identify insensitive and hence resistant lines (6, 74, 76, 78, 124). As the effectors can be used directly by breeders in glasshouse and field trials at any stage in the breeding process, they have been enthusiastically adopted.

There is considerable evidence suggesting that a compatible *Tsn1*-ToxA pathway leading to necrosis is associated with photosynthesis. *Tsn1* expression is regulated by the circadian

clock and light (31), and the *Tsn1*-ToxA interaction is light dependent (66). ToxA is located within the chloroplast and has been reported to bind to plastocyanin (112), a component of photosystem II, and a protein known as ToxABP1 (68). The *Tsn1*-ToxA interaction results in photosystem perturbations (65, 122) leading to disruption of photosynthesis and cell death.

The ToxA system appears to have been acquired by both *S. nodorum* and *P. tritici-repentis* in historic times, within 300 and 80 years, respectively, and appears to operate identically in both pathogens. The ToxA-*Tsn1* interaction has all the hallmarks of a specific effector-receptor interaction; recognition leads to the elaboration of a defense response resulting in cell death. The pathogens subvert the defense response to promote disease development and sporulation.

SnTox2-*Snn2*

The third effector to be identified was SnTox2, another small secreted peptide (7–10 kDa), which is recognized by the dominant host gene *Snn2* on wheat chromosome 2D (35). Molecular markers to identify the *Snn2* genotype have been developed (130). Use of a population segregated for sensitivity to SnTox2 (*Snn2*) and SnToxA (*Tsn1*) enabled the evaluation of the two interactions together. Independently, the SnToxA-*Tsn1* interaction accounted for an average of 20% of the phenotypic variation, whereas the SnTox2-*Snn2* interaction accounted for 49%. Most importantly, the SnToxA-*Tsn1* and SnTox2-*Snn2* interactions were highly additive, showing that multiple NE-host gene interactions generated more disease than a single interaction alone. The same population was used to evaluate the significance of the SnToxA-*Tsn1* and SnTox2-*Snn2* interactions in adult plant disease in the field. This population was evaluated at two locations over two years, and *Tsn1* and *Snn2* were both significantly associated with susceptibility to *S. nodorum* in the field. The SnToxA-*Tsn1* and SnTox2-*Snn2* interactions accounted for 18% and 15%, respectively, showing that NE interactions are important in both the seedling and the adult plant stages. This result indicates that the removal of NE sensitivity genes has the potential to reduce the buildup of disease early in the season (secondary cycles) as well as reduce yield and quality losses due to gain of leaf surface on the upper leaves during grain filling.

SnTox3-*Snn3*

The latest *S. nodorum*–wheat effector system to be identified involves the host gene *Snn3*, an NE sensitivity gene located on wheat chromosome arm 5BS, and the pathogen NE designated SnTox3. The SnTox3-*Snn3* interaction accounted for a significant proportion of the disease phenotype in a wheat population segregating for sensitivity to SnTox3 (*Snn3*), SnToxA (*Tsn1*), and SnTox2 (*Snn2*) but only in isolates with a deleted *SnToxA* gene, showing that the SnToxA-*Tsn1* interaction was epistatic to the SnTox3-*Snn3* interaction. The SnTox2-*Snn2* interaction was also epistatic to the SnTox3-*Snn3* interaction, but the effects of the SnToxA-*Tsn1* and SnTox2-*Snn2* interactions were highly additive (38).

SnTox3 was identified by partial purification and proteomic comparison with the genome assembly (55). *SnTox3* is a 693-bp intron-free gene without obvious similarity to other known genes. *SnTox3* encodes a 230–amino acid preproprotein consisting of a 20–amino acid signal sequence and a predicted prosequence of approximately 30 amino acids, resulting in a mature protein of ~18 kDa. As is reported for many effectors, SnTox3 is cysteine rich with six cysteine residues, each being predicted to be involved in the formation of a disulfide bridge critical to the structure and function of the protein. Approximately 60% of a global collection of field isolates contained *SnTox3*, and the gene is somewhat variable, with 11 different nucleotide haplotypes resulting in four different amino acid sequences. Disruption of the *SnTox3* gene resulted in the elimination of the QTL associated with the *Snn3* locus; however, the other significant QTLs, including that associated with *Snn2*, remained, indicating that

the SnTox3-*Snn3* interaction is independent of other NE interactions. Transformation of the *SnTox3* gene into an avirulent *S. nodorum* isolate also changed this nonpathogen into a pathogen on *Snn3* wheat lines. Additionally, the SnTox3-transformed avirulent isolate was inoculated onto the population, segregating for SnTox3 sensitivity (*Snn3*), and the *Snn3* locus was the only significant QTL detected. The prevalence and importance of ToxA and Tox3 has been compared in Australian wheat cultivars (124). Insensitivity to Tox3 was much rarer than that to ToxA and was associated with a larger gain in disease resistance.

Recently, a second SnTox3 sensitivity was found in *Aegilops tauschii* (the D-genome progenitor of common wheat) and mapped to chromosome arm 5DS (131). Genomic analysis revealed that this gene and *Snn3* are homoeologous and derived from a common ancestor. The *A. tauschii* gene was designated *Snn3-D1* and the wheat 5BS gene designated *Snn3-B1*. *Snn3-D1* is present in numerous *A. tauschii* accessions but not in polyploid wheat, whereas *Snn3-B1* is present in numerous tetraploid (durum) and hexaploid (common) wheat accessions and is derived from *Aegilops speltoides* (B-genome diploid progenitor). *Snn3-B1* confers relatively weaker effects, leading to significantly less necrosis compared with a compatible *Snn3-D1*-SnTox3 interaction.

SnTox4-*Snn4*

A dominant NE sensitivity gene on the short arm of wheat chromosome 1A designated *Snn4* recognizes the NE SnTox4 (2). The SnTox4-*Snn4* interaction accounted for 41% and 24% of the disease variation in the wheat populations Arina × Forno (2) and Salamouni × Katepwa (1), respectively. The SnTox4-*Snn4* interaction is also light dependent, similar to SnToxA-*Tsn1*, SnTox1-*Snn1*, and SnTox2-*Snn2*. SnTox4 is a small proteinaceous molecule estimated to be between 10 and 30 kDa, similar to other known necrotrophic effectors. As with the other reported NE sensitivity genes, molecular markers associated with *Snn4* that are suitable for marker-assisted selection have been developed (1) (**Table 1**).

Recently, winter wheat varieties common in the southeastern United States were evaluated for reactions to the known NEs produced by *S. nodorum* as well as reactions to culture filtrates of isolates collected from the various southeastern regions (26). Several previously uncharacterized NEs and the corresponding host genes were identified. This result indicates that many as yet uncharacterized NE-host gene interactions are present and additional work is needed to evaluate these interactions.

The accumulation of knowledge of necrotrophic effector-host gene interactions has shown that the *S. nodorum*–wheat host pathogen system is largely based on necrotrophic effectors that interact with host gene products in a qualitative (gene-for-gene) fashion but contribute to disease in a quantitative way. This model explains the findings of early studies that showed a quantitative trait made up of many small-effect quantitative loci. Additionally, several reports of additivity are seen in the literature, and this can be explained by the additive nature of NE-host sensitivity gene interactions. In the report of each NE-host gene interaction, several additional QTLs were reported in which the underlying mechanism of resistance/susceptibility is not understood. It is likely that some of these QTLs are indeed as yet unidentified NE-host gene interactions. However, it is also likely that some of these QTLs are not NE-host gene interactions but involve other host mechanisms of resistance.

STAGONOSPORA NODORUM AS A MODEL FOR OTHER PLEOSPORALES

The elucidation of the necrotrophic effector model for the *S. nodorum*–wheat interaction has enabled the rationalization of the complex pattern of multiple, weak QTLs, region-to-region variations, and differences between seedling,

adult leaf, and glume blotch disease. Furthermore, the use of markers linked to NE susceptibility loci or even the NEs themselves has been enthusiastically adopted by breeders.

To what extent does this model apply to other Pleosporales? This group is a rich source of the pathogens expressing what we used to call host-specific toxins. Among these are *P. tritici-repentis*, *Cochliobolus heterostrophus*, and *Alternaria alternata* (127). Pathogenic variation in *S. nodorum* is based on the presence or absence of the known NE genes *SnToxA*, *SnTox1*, and *SnTox3*, which all encode small secreted peptides. *P. tritici-repentis* ToxA appears entirely analogous to the situation in *S. nodorum*. *P. tritici-repentis* also expresses ToxB, another small secreted protein. The *ToxB* gene is present in multiple copies, and virulence is quantitatively related to the number and expression level of these genes (4). Other partially characterized NEs from these species are low-molecular-weight compounds of diverse chemical structures. There is good evidence of proteinaceous secreted effectors in the barley net blotch pathogen *Pyrenophora teres* (86), in the rubber tree pathogen *Corynesopora cassiicola* (10), and in the Ascochyta/Didymella group of legume pathogens (J. Lichtenzveig, F. Kessie, R.M. Shah, R.P. Oliver, unpublished results). A common feature of this group is that effectors can normally be recovered from culture filtrates. In stark contrast to the dominant NE model applicable to *S. nodorum*, the closely related canola pathogen *L. maculans* produces negatively active proteinaceous effectors (84, 121).

All of these pathogens have caused recent devastating epidemics of crops, and all of the effector genes have characteristics consistent with having been acquired by horizontal gene transfer (79). These data suggest that the Pleosporales are a rapidly evolving group, adapting to new crops and new agricultural practices. Their success may be related to a propensity to acquire genes by horizontal transfer that can subvert the host defense machinery to cause disease.

SUMMARY POINTS

1. *S. nodorum* is a model for necrotrophic Pleosporales pathogens.
2. Genetic resistance is quantitative and governed by multiple, mostly weak, QTLs.
3. Genome sequencing has permitted genetic dissection of pathogenicity genes and revealed the critical role of NEs.
4. Functional genomics has identified the role of signaling genes and key biochemical pathways in pathogenesis.
5. Three NEs have been fully characterized. All are small secreted proteins that induce necrosis in specific genotypes of wheat.
6. Global variation in populations of the pathogen is consistent with rapid gene flow over continental distances.
7. Genes for NEs are irregularly distributed in populations.
8. NEs are being used by breeders to produce resistant varieties of wheat.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors acknowledge the support of the Australian Grains Research and Development Corporation, the Australian Research Council, USDA-NIFA AFRI Microbial Biology Program competitive grant 2009-04265, USDA-ARS CRIS 5442-22000-043-09G, and National Research Initiative competitive grant 2008-35301-19248 from the USDA National Institute of Food and Agriculture. Space constraints prevent a comprehensive appraisal of all relevant literature. We apologize to colleagues whose papers are not cited directly.

LITERATURE CITED

1. Abeysekara NS, Faris JD, Chao S, McClean PE, Friesen TL. 2012. Whole genome QTL analysis of *Stagonospora nodorum* blotch resistance and validation of the SnTox4-Snn4 interaction in hexaploid wheat. *Phytopathology* 102:94–104
2. Abeysekara NS, Friesen TL, Keller B, Faris JD. 2009. Identification and characterization of a novel host-toxin interaction in the wheat–*Stagonospora nodorum* pathosystem. *Theor. Appl. Genet.* 120:117–26
3. Akagi Y, Akamatsu H, Otani H, Kodama M. 2009. Horizontal chromosome transfer, a mechanism for the evolution and differentiation of a plant-pathogenic fungus. *Eukaryot. Cell* 8:1732–38
4. Amaike S, Ozga JA, Basu U, Strelkov SE. 2008. Quantification of *ToxB* gene expression and formation of appressoria by isolates of *Pyrenophora tritici-repentis* differing in pathogenicity. *Plant Pathol.* 57:623–33
5. Anderson JA, Effertz RJ, Faris JD, Franel LJ, Meinhardt SW, Gill BS. 1999. Genetic analysis of sensitivity to a *Pyrenophora tritici-repentis* necrosis-inducing toxin in durum and common wheat. *Phytopathology* 89:293–97
6. Antoni EA, Rybak K, Tucker MP, Hane JK, Solomon PS, et al. 2010. Ubiquity of ToxA and absence of ToxB in Australian populations of *Pyrenophora tritici-repentis*. *Australas. Plant Pathol.* 39:63–68
7. Arraiano LS, Balaam N, Fenwick PM, Chapman C, Feuerhelm D, et al. 2009. Contributions of disease resistance and escape to the control of *Septoria tritici* blotch of wheat. *Plant Pathol.* 58:910–22
8. Ballance GM, Lamari L, Bernier CC. 1989. Purification and characterization of a host-selective necrosis toxin from *Pyrenophora tritici-repentis*. *Physiol. Mol. Plant Pathol.* 35:203–13
9. Ballance GM, Lamari L, Bernier CC. 1996. Cloning expression and occurrence of the gene encoding the Ptr necrosis toxin from *Pyrenophora tritici-repentis*. *Mol. Plant Pathol. Online*. **http://www.bspp.org.uk/mppol/1996/1209ballance**
10. Barthe P, Pujade-Renaud V, Breton F, Gargani D, Thai R, et al. 2007. Structural analysis of cassiicolin, a host-selective protein toxin from *Corynespora cassiicola*. *J. Mol. Biol.* 367:89–101
11. Bearchell SJ, Fraaije BA, Shaw MW, Fitt BDL. 2005. Wheat archive links long-term fungal pathogen population dynamics to air pollution. *Proc. Natl. Acad. Sci. USA* 102:5438–42
12. Bennett RS, Milgroom MG, Sainudiin R, Cunfer BM, Bergstrom GC. 2007. Relative contribution of seed-transmitted inoculum to foliar populations of *Phaeosphaeria nodorum*. *Phytopathology* 97:584–91
13. Bhathal JS, Loughman R. 2001. Ability of retained stubble to carry-over leaf diseases of wheat in rotation crops. *Aust. J. Exp. Agric.* 41:649–53
14. Blixt E, Djurle A, Yuen J, Olson Ã. 2009. Fungicide sensitivity in Swedish isolates of *Phaeosphaeria nodorum*. *Plant Pathol.* 58:655–64
15. Blixt E, Olson Å, Högberg N, Djurle A, Yuen J. 2008. Mating type distribution and genetic structure are consistent with sexual recombination in the Swedish population of *Phaeosphaeria nodorum*. *Plant Pathol.* 57:634–41
16. Bringans S, Hane JK, Casey T, Tan KC, Lipscombe R, et al. 2009. Deep proteogenomics: high throughput gene validation by multidimensional liquid chromatography and mass spectrometry of proteins from the fungal wheat pathogen *Stagonospora nodorum*. *BMC Bioinforma.* 10:301
17. Casey T, Solomon PS, Bringans S, Tan KC, Oliver RP, Lipscombe R. 2010. Quantitative proteomic analysis of G-protein signalling in *Stagonospora nodorum* using isobaric tags for relative and absolute quantification. *Proteomics* 10:38–47

18. Caten CE, Newton AC. 2000. Variation in cultural characteristics, pathogenicity, vegetative compatibility and electrophoretic karyotype within field populations of *Stagonospora nodorum*. *Plant Pathol.* 49:219–26
19. Chisholm ST, Coaker G, Day B, Staskawicz BJ. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* 124:803–14
20. Chu C-G, Faris JD, Xu S, Friesen TL. 2010. Genetic analysis of disease susceptibility contributed by the compatible Tsn1-SnToxA and Snn1-SnTox1 interactions in the wheat–*Stagonospora nodorum* pathosystem. *Theor. Appl. Genet.* 129:1451–59
21. Ciuffetti LM, Tuori RP. 1999. Advances in the characterization of the *Pyrenophora tritici-repentis*–wheat interaction. *Phytopathology* 89:444–49
22. Ciuffetti LM, Tuori RP, Gaventa JM. 1997. A single gene encodes a selective toxin causal to the development of tan spot of wheat. *Plant Cell* 9:135–44
23. Clutterbuck JA. 2011. Genomic evidence of repeat-induced point mutation (RIP) in filamentous ascomycetes. *Fungal Genet. Biol.* 48:306–26
24. Cooley R, Shaw R, Franklin F, Caten C. 1988. Transformation of the phytopathogenic fungus *Septoria nodorum* to hygromycin B resistance. *Curr. Genet.* 13:383–86
25. Cooley RN, Caten CE. 1991. Variation in electrophoretic karyotype between strains of *Septoria nodorum*. *Mol. Gen. Genet.* 228:17–23
26. Crook AD, Friesen TL, Liu ZH, Ojiambo PS, Cowger C. 2012. Novel necrotrophic effectors from *Stagonospora nodorum* and corresponding host genes in winter wheat germplasm in the southeastern US. *Phytopathology* 102:498–505
27. Dickman MB, Park YK, Oltersdorf T, Li W, Clemente T, French R. 2001. Abrogation of disease development in plants expressing animal antiapoptotic genes. *Proc. Natl. Acad. Sci. USA* 98:6957–62
28. Ellwood SR, Liu Z, Syme RA, Lai Z, Hane JK, et al. 2010. A first genome assembly of the barley fungal pathogen *Pyrenophora teres* f. *teres*. *Genome Biol.* 11:R109
29. Faris JD, Anderson JA, Francl LJ, Jordahl JG. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. *Phytopathology* 86:459–63
30. Faris JD, Friesen TL. 2009. Reevaluation of a tetraploid wheat population indicates that the Tsnl-ToxA interaction is the only factor governing *Stagonospora nodorum* blotch susceptibility. *Phytopathology* 99:906–12
31. Faris JD, Zhang Z, Lu H, Lu S, Reddy L, et al. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proc. Natl. Acad. Sci. USA* 107:13544–49
32. Faris JD, Zhang Z, Rasmussen JB, Friesen TL. 2011. Variable expression of the *Stagonospora nodorum* effector SnToxA among isolates is correlated with levels of disease susceptibility in wheat. *Mol. Plant-Microbe Interact.* 24:1419–26
33. Feng J, Li W, Hwang SF, Gossen BD, Strelkov SE. 2012. Enhanced gene replacement frequency in KU70 disruption strain of *Stagonospora nodorum*. *Microbiol. Res.* 167:173–78
34. Friesen TL, Faris JD, Solomon PS, Oliver RP. 2008. Host-specific toxins: effectors of necrotrophic pathogenicity. *Cell. Microbiol.* 10:1421–28
35. Friesen TL, Meinhardt SW, Faris JD. 2007. The *Stagonospora nodorum*–wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. *Plant J.* 51:681–92
36. Friesen TL, Rasmussen JB, Kwon CY, Ali S, Francl LJ, Meinhardt SW. 2002. Reaction of Ptr ToxA-insensitive wheat mutants to *Pyrenophora tritici-repentis* race 1. *Phytopathology* 92:38–42
37. Friesen TL, Stukenbrock EH, Liu Z, Meinhardt S, Ling H, et al. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. *Nat. Genet.* 38:953–56
38. Friesen TL, Zhang Z, Solomon PS, Oliver RP, Faris JD. 2008. Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. *Plant Physiol.* 146:682–93
39. Fudal I, Ross S, Brun H, Besnard AL, Ermel M, et al. 2009. Repeat-induced point mutation (RIP) as an alternative mechanism of evolution toward virulence in *Leptosphaeria maculans*. *Mol. Plant-Microbe Interact.* 22:932–41

40. Haen KM, Lu H, Friesen TL, Faris JD. 2004. Genomic targeting and high-resolution mapping of the *Tsn1* gene in wheat. *Crop Sci.* 44:951–62
41. Hane JK, Lowe RG, Solomon PS, Tan KC, Schoch CL, et al. 2007. Dothideomycete plant interactions illuminated by genome sequencing and EST analysis of the wheat pathogen *Stagonospora nodorum*. *Plant Cell* 19:3347–68
42. Hane JK, Oliver RP. 2008. RIPCAL: a tool for alignment-based analysis of repeat-induced point mutations in fungal genomic sequences. *BMC Bioinforma.* 9:478
43. Hane JK, Oliver RP. 2010. In silico reversal of repeat-induced point mutation (RIP) identifies the origins of repeat families and uncovers obscured duplicated genes. *BMC Genomics* 11:655
44. Hane JK, Rouxel T, Howlett B, Kema G, Goodwin S, Oliver R. 2011. A novel mode of chromosomal evolution peculiar to filamentous Ascomycete fungi. *Genome Biol.* 12:R45
45. Hane JK, Williams A, Oliver RP. 2011. Genomic and comparative analysis of the class Dothideomycetes. In *The Mycota*, Vol. 14, ed. S Poggeler, J Wostemeyer, pp. 205–26. Berlin: Springer-Verlag
46. Idnurm A, Howlett BJ. 2002. Isocitrate lyase is essential for pathogenicity of the fungus *Leptosphaeria maculans* to canola (*Brassica napus*). *Eukaryot. Cell* 1:719–24
47. Ipcho SV, Hane JK, Ahren D, Friesen TL, Solomon PS, Oliver RP. 2012. Comprehensive transcriptomic analysis of the wheat pathogen *Stagonospora nodorum*: gene model validation, effector candidate genes and intensive host regulation of metabolism. *Mol. Plant Pathol.* doi: 10.1111/j.1364-3703.2011.00770.x
48. Ipcho SVS, Tan KC, Koh G, Gummer J, Oliver RP, et al. 2010. The transcription factor StuA regulates central carbon metabolism, mycotoxin production, and effector gene expression in the wheat pathogen *Stagonospora nodorum*. *Eukaryot. Cell* 9:1100–8
49. Jones JDG, Dangl JL. 2006. The plant immune system. *Nature* 444:323–29
50. Keller SM, McDermott JM, Pettway RE, Wolfe MS, McDonald BA. 1997. Gene flow and sexual reproduction in the wheat glume blotch pathogen *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*). *Phytopathology* 87:353–58
51. Keller SM, Wolfe MS, McDermott JM, McDonald BA. 1997. High genetic similarity among populations of *Phaeosphaeria nodorum* across wheat cultivars and regions in Switzerland. *Phytopathology* 87:1134–39
52. Lamari L, Bernier CC. 1989. Toxin of *Pyrenophora tritici-repentis*: host-specificity, significance in disease, and inheritance of host-reaction. *Phytopathology* 79:740–44
53. Lamari L, Bernier CC. 1991. Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. *Phytopathology* 81:1092–95
54. Lewis DH. 1973. Concepts in fungal nutrition and the origin of biotrophy. *Biol. Rev.* 48:261–78
55. Liu Z, Faris JD, Oliver RP, Tan KC, Solomon PS, et al. 2009. SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. *PLoS Pathog.* 5(9):e1000581
56. Liu Z, Friesen TL, Ling H, Meinhardt SW, Oliver RP, et al. 2006. The *Tsn1*-ToxA interaction in the wheat–*Stagonospora nodorum* pathosystem parallels that of the wheat–tan spot system. *Genome* 49:1265–73
57. Liu Z-H, Zhang Z, Faris JD, Oliver RP, Syme R, et al. 2012. The cysteine rich necrotrophic effector SnTox1 produced by *Stagonospora nodorum* triggers susceptibility of wheat lines harboring Snn1. *PLoS Pathog.* 8(1):e1002467
58. Liu ZH, Faris JD, Meinhardt SW, Ali S, Rasmussen JB, Friesen TL. 2004. Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin produced by *Stagonospora nodorum*. *Phytopathology* 94:1056–60
59. Liu ZH, Friesen TL, Rasmussen JB, Ali S, Meinhardt SW, Faris JD. 2004. Quantitative trait loci analysis and mapping of seedling resistance to *Stagonospora nodorum* leaf blotch in wheat. *Phytopathology* 94:1061–67
60. Lowe RG, Lord M, Rybak K, Trengove RD, Oliver RP, Solomon PS. 2008. A metabolomic approach to dissecting osmotic stress in the wheat pathogen *Stagonospora nodorum*. *Fungal Genet. Biol.* 45:1479–86
61. Lowe RGT, Lord M, Rybak K, Trengove RD, Oliver RP, Solomon PS. 2009. Trehalose biosynthesis is involved in sporulation of *Stagonospora nodorum*. *Fungal Genet. Biol.* 46:381–89
62. Lu H, Faris JD. 2006. Macro- and microcolinearity between the genomic region of wheat chromosome 5B containing the *Tsn1* gene and the rice genome. *Funct. Integr. Genomics* 6:90–103

63. Malkus A, Song Q, Cregan P, Arseniuk E, Ueng PP. 2009. Genetic linkage map of *Phaeosphaeria nodorum*, the causal agent of stagonospora nodorum blotch disease of wheat. *Eur. J. Plant Pathol.* 124:681–90
64. Manning VA, Andrie RM, Trippe AF, Ciuffetti LM. 2004. Ptr ToxA requires multiple motifs for complete activity. *Mol. Plant-Microbe Interact.* 17:491–501
65. Manning VA, Chu AL, Steeves JE, Wolpert TJ, Ciuffetti LM. 2009. A host-selective toxin of *Pyrenophora tritici-repentis*, Ptr ToxA, induces photosystem changes and reactive oxygen species accumulation in sensitive wheat. *Mol. Plant-Microbe Interact.* 22:665–76
66. Manning VA, Ciuffetti LM. 2005. Localization of Ptr ToxA produced by *Pyrenophora tritici-repentis* reveals protein import into wheat mesophyll cells. *Plant Cell* 17:3203–12
67. Manning VA, Hamilton SM, Karplus PA, Ciuffetti LM. 2008. The Arg-Gly-Asp-containing, solvent-exposed loop of Ptr ToxA is required for internalization. *Mol. Plant-Microbe Interact.* 21:315–25
68. Manning VA, Hardison LK, Ciuffetti LM. 2007. Ptr ToxA interacts with a chloroplast-localized protein. *Mol. Plant-Microbe Interact.* 20:168–77
69. McDonald BA, Miles J, Nelson LR, Pettway RE. 1994. Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. *Phytopathology* 84:250–55
70. McDonald BA, Zhan J, Yarden O, Hogan K, Garton J, Pettway RE. 1999. The population genetics of *Mycosphaerella graminicola* and *Stagonospora nodorum*. In *Septoria on Cereals: A Study of Pathosystems*, ed. J Lucas, P Bowyer, HM Anderson, pp. 44–69. Oxfordshire, UK: CABI
71. Meinhardt SW, Cheng W, Kwon CY, Donohue CM, Rasmussen JB. 2002. Role of the arginyl-glycyl-aspartic motif in the action of Ptr ToxA produced by *Pyrenophora tritici-repentis*. *Plant Physiol.* 130:1545–51
72. Mercado Vergnes D, Zhanarbekova A, Renard ME, Duveiller E, Maraite H. 2006. Mating types of *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*) from Central Asia. *J. Phytopathol.* 154:317–19
73. Murray GM, Brennan JP. 2009. Estimating disease losses to the Australian wheat industry. *Australas. Plant Pathol.* 38:558–70
74. Oliver RP. 2008. Plant breeding for disease resistance in the age of effectors. *Phytoparasitica* 37:1–5
75. Oliver RP, Ipcho SVS. 2004. *Arabidopsis* pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. *Mol. Plant Pathol.* 5:347–52
76. Oliver RP, Lord M, Rybak K, Faris JD, Solomon PS. 2008. Emergence of tan spot disease caused by toxigenic *Pyrenophora tritici-repentis* in Australia is not associated with increased deployment of toxin-sensitive cultivars. *Phytopathology* 98:488–91
77. Oliver RP, Rybak K, Shankar M, Loughman R, Harry N, Solomon PS. 2008. Quantitative disease resistance assessment by real-time PCR using the *Stagonospora nodorum*–wheat pathosystem as a model. *Plant Pathol.* 57:527–32
78. Oliver RP, Rybak K, Solomon PS, Ferguson-Hunt M. 2009. Prevalence of ToxA-sensitive alleles of the wheat gene *Tsn1* in Australian and Chinese wheat cultivars *Crop Pasture Sci.* 60:348–52
79. Oliver RP, Solomon PS. 2008. Recent fungal diseases of crop plants: Is lateral gene transfer a common theme? *Mol. Plant-Microbe Interact.* 21:287–93
80. Oliver RP, Solomon PS, Hane J, Ferguson-Hunt M, McDonald BA, et al. 2008. Multiple host-specific toxins, lateral gene transfer and gene loss in the evolution of cereal Pleosporalean pathogens. *Proc. 9th Int. Congr. Plant Pathol.*, *Torino*, *Italy*, *Aug. 24–29* **http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=216140**
81. Rawson J. 2000. *Transposable elements in the phytopathogenic fungus Stagonospora nodorum.* PhD thesis. Univ. Birmingham, Birmingham, UK. 270 pp.
82. Reddy L, Friesen TL, Meinhardt SW, Chao S, Faris JD. 2008. Genomic analysis of the *Snn1* locus on wheat chromosome arm 1BS and the identification of candidate genes. *Plant Genome* 1:55–66
83. Richards TA. 2011. Genome evolution: horizontal movements in the fungi. *Curr. Biol.* 21:R166–68
84. Rouxel T, Grandaubert J, Hane J, Hoede C, van de Wouw A, et al. 2011. The compartmentalized genome of *Leptosphaeria maculans*: diversification of effectors within genomic regions affected by repeat induced point mutations. *Nat. Commun.* 2: 202
85. Sarma GN, Manning VA, Ciuffetti LM, Karplus PA. 2005. Structure of Ptr ToxA: an RGD-containing host-selective toxin from *Pyrenophora tritici-repentis*. *Plant Cell* 17:3190–202
86. Sarpeleh A, Wallwork H, Tate ME, Catcheside DEA, Able AJ. 2008. Initial characterisation of phytotoxic proteins isolated from *Pyrenophora teres*. *Physiol. Mol. Plant Pathol.* 72:73–79

87. Schmitt I, Lumbsch HT. 2009. Ancient horizontal gene transfer from bacteria enhances biosynthetic capabilities of fungi. *PLoS ONE* 4:e4437
88. Schoch CL, Crous PW, Groenewald JZ, Boehm EW, Burgess TI, et al. 2009. A class-wide phylogenetic assessment of Dothideomycetes. *Stud. Mycol.* 64:1–15S10
89. Selker EU. 1997. Epigenetic phenomena in filamentous fungi: useful paradigms or repeat-induced confusion? *Trends Genet.* 13:296–301
90. Shaw MW, Bearchell SJ, Fitt BDL, Fraaije BA. 2008. Long-term relationships between environment and abundance in wheat of *Phaeosphaeria nodorum* and *Mycosphaerella graminicola*. *New Phytol.* 177:229–38
91. Solomon PS, Jörgens CI, Oliver RP. 2006. δ-aminolaevulinic acid synthesis is required for virulence of the wheat pathogen *Stagonospora nodorum*. *Microbiology* 152:1533–38
92. Solomon PS, Lee RC, Wilson TJG, Oliver RP. 2004. Pathogenicity of *Stagonospora nodorum* requires malate synthase. *Mol. Microbiol.* 53:1065–73
93. Solomon PS, Lowe RGT, Tan KC, Waters ODC, Oliver RP. 2006. *Stagonospora nodorum*: cause of *Stagonospora nodorum* blotch of wheat. *Mol. Plant Pathol.* 7:147–56
94. Solomon PS, Lowe RGT, Trengove RD, Rechberger J, Oliver RP. 2006. Normalization of metabolites in heterogenous systems using genomics. *Anal. Biochem.* 350:156–58
95. Solomon PS, Oliver RP. 2004. Functional characterisation of glyoxalase I from the fungal wheat pathogen *Stagonospora nodorum*. *Curr. Genet.* 46:115–21
96. Solomon PS, Parker K, Loughman R, Oliver RP. 2004. Both mating types of *Phaeosphaeria* (anamorph *Stagonospora*) *nodorum* are present in Western Australia. *Eur. J. Plant Pathol.* 110:763–66
97. Solomon PS, Rybak K, Trengove RD, Oliver RP. 2006. Investigating the role of calcium/calmodulin-dependent protein kinases in *Stagonospora nodorum*. *Mol. Microbiol.* 62:367–81
98. Solomon PS, Tan K-C, Oliver RP. 2005. Mannitol 1-phosphate metabolism is required for sporulation in planta of the wheat pathogen *Stagonospora nodorum*. *Mol. Plant-Microbe Interact.* 18:110–15
99. Solomon PS, Tan KC, Sanchez P, Cooper RM, Oliver RP. 2004. The disruption of a Gα subunit sheds new light on the pathogenicity of *Stagonospora nodorum* on wheat. *Mol. Plant-Microbe Interact.* 17:456–66
100. Solomon PS, Thomas SW, Spanu P, Oliver RP. 2003. The utilisation of di/tripeptides by *Stagonospora nodorum* is dispensable for wheat infection. *Physiol. Mol. Plant Pathol.* 63:191–99
101. Solomon PS, Waters ODC, Jörgens CI, Lowe RGT, Rechberger J, et al. 2006. Mannitol is required for asexual sporulation in the wheat pathogen *Stagonospora nodorum* (glume blotch). *Biochem. J.* 399:231–39
102. Solomon PS, Waters ODC, Oliver RP. 2007. Decoding the mannitol enigma in filamentous fungi. *Trends Microbiol.* 15:257–62
103. Solomon PS, Waters ODC, Simmonds J, Cooper RM, Oliver RP. 2005. The Mak2 MAP kinase signal transduction pathway is required for pathogenicity in *Stagonospora nodorum*. *Curr. Genet.* 48:60–68
104. Solomon PS, Wilson TJG, Rybak K, Parker K, Lowe RGT, Oliver RP. 2006. Structural characterisation of the interaction between *Triticum aestivum* and the dothideomycete pathogen *Stagonospora nodorum*. *Eur. J. Plant Pathol.* 114:275–82
105. Sommerhalder RJ, McDonald BA, Mascher F, Zhan J. 2010. Sexual recombinants make a significant contribution to epidemics caused by the wheat pathogen *Phaeosphaeria nodorum*. *Phytopathology* 100:855–62
106. Sommerhalder RJ, McDonald BA, Mascher F, Zhan J. 2011. Effect of hosts on competition among clones and evidence of differential selection between pathogenic and saprophytic phases in experimental populations of the wheat pathogen *Phaeosphaeria nodorum*. *BMC Evol. Biol.* 11:188
107. Sommerhalder RJ, McDonald BA, Zhan J. 2006. The frequencies and spatial distribution of mating types in *Stagonospora nodorum* are consistent with recurring sexual reproduction. *Phytopathology* 96:234–39
108. Sommerhalder RJ, McDonald BA, Zhan J. 2007. Concordant evolution of mitochondrial and nuclear genomes in the wheat pathogen *Phaeosphaeria nodorum*. *Fungal Genet. Biol.* 44:764–72
109. Stukenbrock EH, Banke S, McDonald BA. 2006. Global migration patterns in the fungal wheat pathogen *Phaeosphaeria nodorum*. *Mol. Ecol.* 15:2895–904
110. Stukenbrock EH, Banke S, Zala M, McDonald BA, Oliver RP. 2005. Isolation and characterization of EST-derived microsatellite loci from the fungal wheat pathogen *Phaeosphaeria nodorum*. *Mol. Ecol. Notes* 5:931–33

111. Stukenbrock EH, McDonald BA. 2007. Geographical variation and positive diversifying selection in the host-specific toxin SnToxA. *Mol. Plant Pathol.* 8:321–32
112. Tai YS, Bragg J, Meinhardt SW. 2007. Functional characterization of ToxA and molecular identification of its intracellular targeting protein in wheat. *Am. J. Plant Physiol.* 2:76–89
113. Tan K-C, Heazlewood JL, Millar AH, Oliver RP, Solomon PS. 2009. Proteomic identification of extracellular proteins regulated by the *Gna1* Gα subunit in *Stagonospora nodorum*. *Mycol. Res.* 113:523–31
114. Tan KC, Heazlewood JL, Millar AH, Thomson G, Oliver RP, Solomon PS. 2008. A signaling-regulated, short-chain dehydrogenase of *Stagonospora nodorum* regulates asexual development. *Eukaryot. Cell* 7:1916–29
115. Tan KC, Ipcho SVS, Trengove RD, Oliver RP, Solomon PS. 2009. Assessing the impact of transcriptomics, proteomics and metabolomics on fungal phytopathology. *Mol. Plant Pathol.* 10:703–15
116. Tan KC, Trengove RD, Maker GL, Oliver RP, Solomon PS. 2009. Metabolite profiling identifies the mycotoxin alternariol in the pathogen *Stagonospora nodorum*. *Metabolomics* 5:330–35
117. Thines E, Weber RW, Talbot NJ. 2000. MAP kinase and protein kinase A-dependent mobilization of triacylglycerol and glycogen during appressorium turgor generation by *Magnaporthe grisea*. *Plant Cell* 12:1703–18
118. Tomás A, Bockus WW. 1987. Cultivar-specific toxicity of culture filtrates of *Pyrenophora tritici-repentis*. *Phytopathology* 77:1337–40
119. Tomás A, Feng GH, Reeck GR, Bockus WW, Leach JE. 1990. Purification of a cultivar-specific toxin from *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. *Mol. Plant-Microbe Interact.* 3:221–24
120. Tuori RP, Wolpert TJ, Ciuffetti LM. 1995. Purification and immunological characterization of toxic components from cultures of *Pyrenophora tritici-repentis*. *Mol. Plant-Microbe Interact.* 8:41–48
121. Van de Wouw AP, Cozijnsen AJ, Hane JK, Brunner PC, McDonald BA, et al. 2010. Evolution of linked avirulence effectors in *Leptosphaeria maculans* is affected by genomic environment and exposure to resistance genes in host plants. *PLoS Pathog.* 6:e1001180
122. Vincent D, Du Fall LA, Livk A, Mathesius U, Lipscombe RJ, et al. 2011. A functional genomics approach to dissect the mode of action of the *Stagonospora nodorum* effector protein SnToxA in wheat. *Mol. Plant Pathol.* 13:467–82
123. Walton JD. 2000. Horizontal gene transfer and the evolution of secondary metabolite gene clusters in fungi: an hypothesis. *Fungal Genet. Biol.* 30:167–71
124. Waters ODC, Lichtenzveig J, Rybak K, Friesen TL, Oliver RP. 2011. Prevalence and importance of sensitivity to the *Stagonospora nodorum* necrotrophic effector SnTox3 in current western Australian wheat cultivars. *Crop Pasture Sci.* 62:556–62
125. Wilson RA, Gibson RP, Quispe CF, Littlechild JA, Talbot NJ. 2010. An NADPH-dependent genetic switch regulates plant infection by the rice blast fungus. *Proc. Natl. Acad. Sci. USA* 107:21902–7
126. Wilson RA, Jenkinson JM, Gibson RP, Littlechild JA, Wang ZY, Talbot NJ. 2007. Tps1 regulates the pentose phosphate pathway, nitrogen metabolism and fungal virulence. *EMBO J.* 26:3673–85
127. Wolpert TJ, Dunkle LD, Ciuffetti LM. 2002. Host-selective toxins and avirulence determinants: What's in a name? *Annu. Rev. Phytopathol.* 40:251–85
128. Xu SS, Friesen TL, Mujeeb-Kazi A. 2004. Seedling resistance to tan spot and *Stagonospora nodorum* blotch in synthetic hexaploid wheats. *Crop Sci.* 44:2238–45
129. Zhang HF, Francl LJ, Jordahl JG, Meinhardt SW. 1997. Structural and physical properties of a necrosis-inducing toxin from *Pyrenophora tritici-repentis*. *Phytopathology* 87:154–60
130. Zhang Z, Friesen TL, Simons KJ, Xu SS, Faris JD. 2009. Development, identification, and validation of markers for marker-assisted selection against the *Stagonospora nodorum* toxin sensitivity genes *Tsn1* and *Snn2* in wheat. *Mol. Breed.* 23:35–49
131. Zhang Z, Friesen TL, Xu SS, Shi G, Liu Z, et al. 2011. Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to *Stagonospora nodorum*. *Plant J.* 65:27–38

Annual Review of Phytopathology

Volume 50, 2012

Contents

Errata

An online log of corrections to *Annual Review of Phytopathology* articles may be found at http://phyto.annualreviews.org/